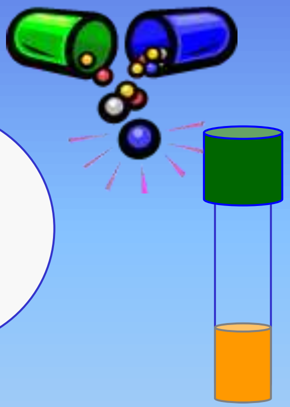
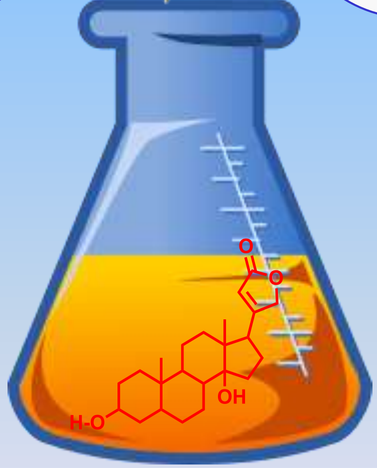
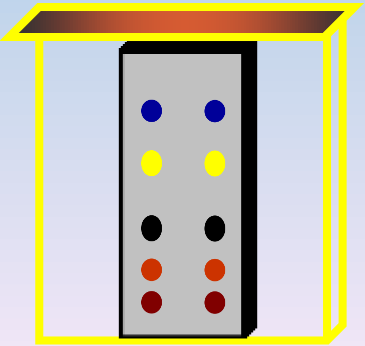
NATURAL PRODUCTS & QUALITY CONTROL
(208)

Cairo University
Faculty of Pharmacy

PRELAB. NO. 1







PHYTOCHEMICAL SCREENING

What do you want to be after graduation?

☐ Scientist discovering new medicines.

☐ Quality Control Pharmacist.

☐ Forensic medicine Pharmacist.

☐ Manufacturing Plant Owner.

☐ Treat Patients with herbal medicine.

☐ Pharmacist in public Pharmacy.

We are preparing you to be any of the above.

PHYTOCHEMICAL SCREENING

PHYTOCHEMICAL SCREENING

WATER EXTRACT

Powder + Water
Macerate for about 10minutes, filter

Test for
Carbohydrates &/or glycosides, Tannins, Saponins and Enzymes

70% ALCOHOL EXTRACT

Powder + 70% alcohol
Macerate for about 20 minutes , frequent shaking and filter

Test for
Sterols &/or Triterpenes, Cardiac glycosides, Alkaloids and Anthraquinones

1% HCL EXTRACT

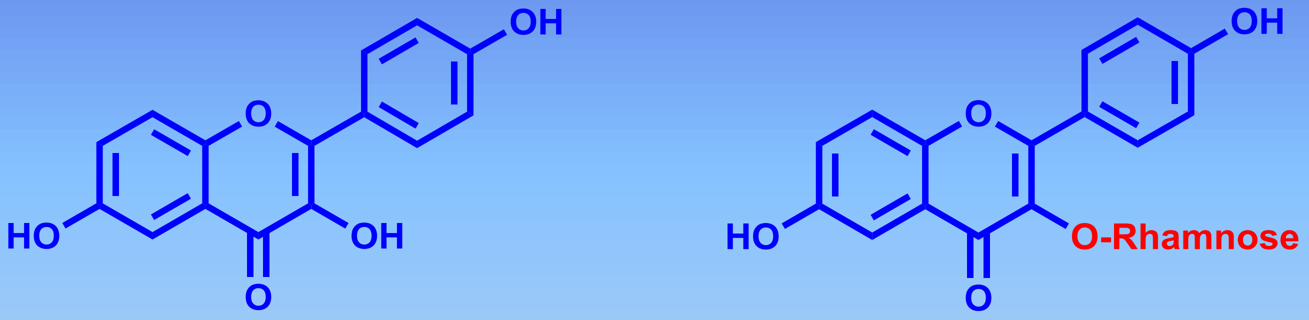
Powder + 1% HCl
Vigorous shaking and filter

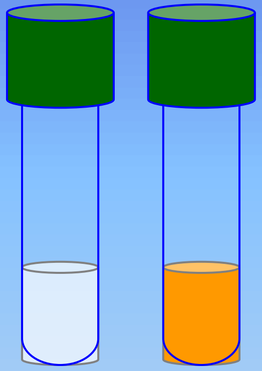
Test for
Flavonoids

(A) TESTS CARRIED OUT ON 1% HCL EXTRACT

1% HCL EXTRACT FILTERED THROUGH COTTON

10%NaOH test : General Test for free and/or combined flavonoids



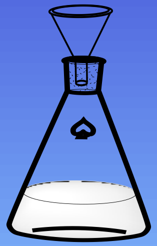


2 ml Extract + 5ml 10% NaOH → yellow colour

N.B : Compare with blank (5ml water + 2ml extract)

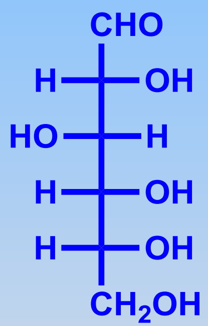
(B) TESTS CARRIED OUT ON WATER EXTRACT

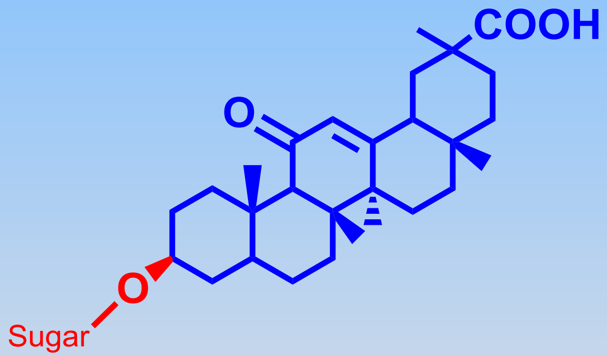
WATER EXTRACT FILTERED THROUGH COTTON

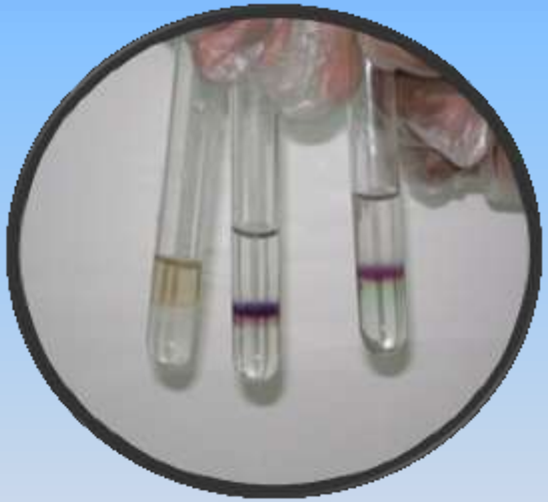


1) Test for carbohydrates and/or glycosides

Molish's test







2ml water extract + 1ml alc. α-naphthol (Shake) + 2ml conc. H_2SO_4 on the wall of test tube

→ violet ring.

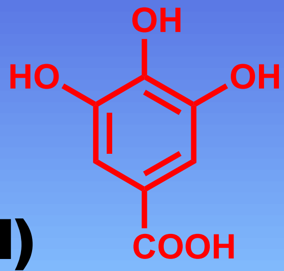
(A) TESTS CARRIED OUT ON WATER EXTRACT

2) Test for tannins (FeCl$_3$ Test)

2ml Water extract + 2ml FeCl$_3$ test solution

Black Green Colour
(Condensed - Catechol)

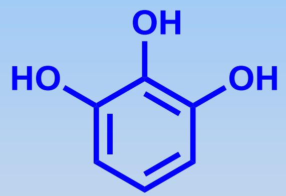
Blue Green Colour
Hydrolysable - pyrogallol)

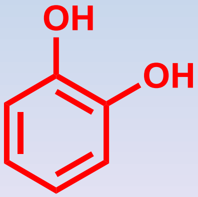


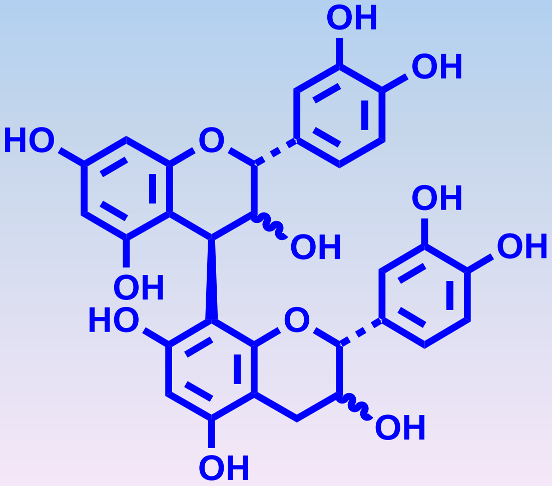
N.B : Blank is done

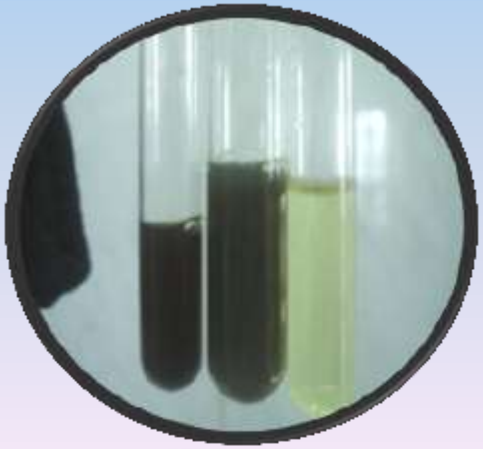
B1→(2ml Water + 2ml FeCl$_3$)

B2→ (2ml water extract + 2ml water)



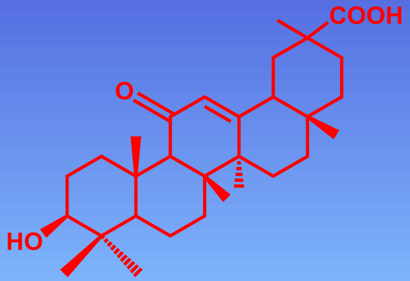


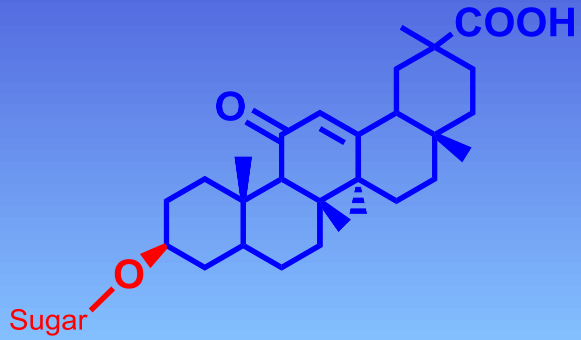


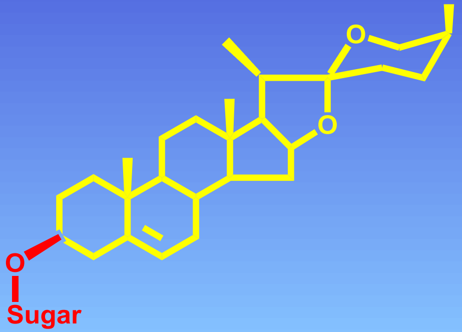


(A) TESTS CARRIED OUT ON WATER EXTRACT

3) Test for saponins

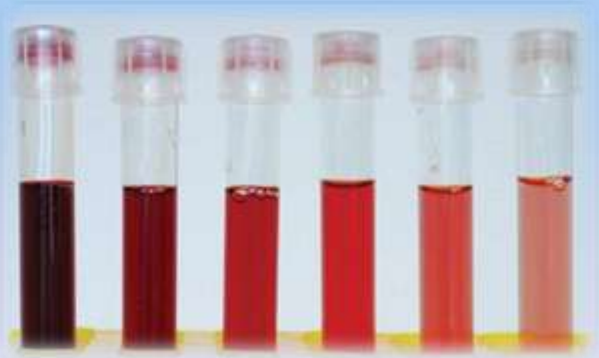






a. Froth test:

Shake vigorously water extract & allow to stand persistent froth (1 cm or more) for 5 min.



b. Haemolysis test:

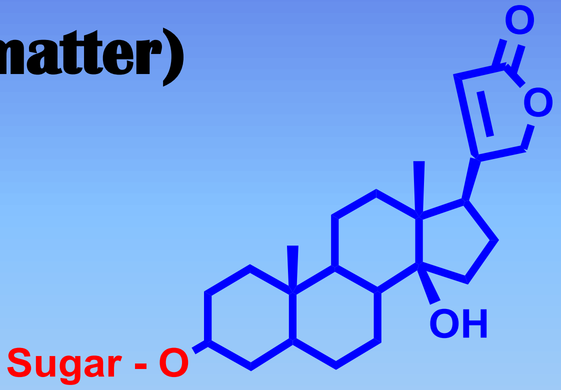
Saponins causes haemolysis for RBCs in isotonic solution (opaque → transparent).

(C) TESTS CARRIED OUT ON ALCOHOL EXTRACT

1) Test for cardiac glycosides

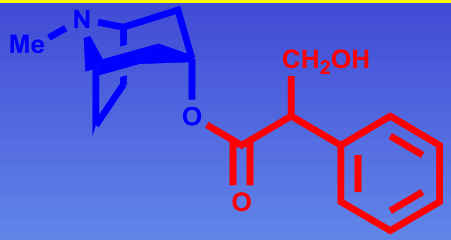
Baljet test (For 5 membered lactone ring - Cardenolides)

- ☐ 5ml Alc. Extract + 5ml lead acetate (to ppt. tannins & colouring matter)
- ☐ Filter



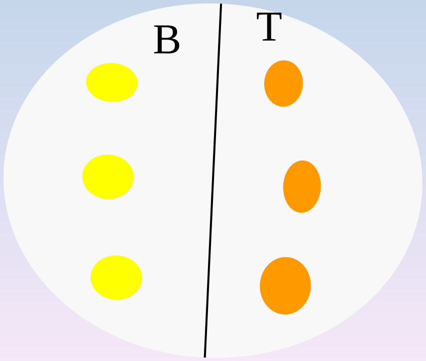
- ☐ Add 5ml Na_2HPO_4 (to ppt. excess lead) then filter
- ☐ The filtrate should be clear & colourless
- ☐ 2ml Filtrate + 2ml Baljet reagent (Picric acid + 10% NaOH), allow to stand for 20 min (for development of colour)
- ☐ Compare the colour by nesslerization with blank (water + Baljet reagent)
- ☐ Orange – red colour indicate presence of cardiac glycosides.

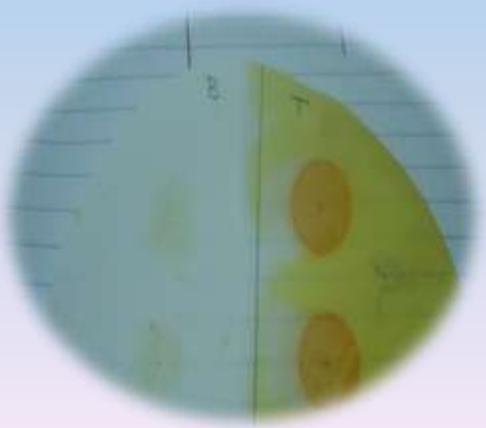
(C) TESTS CARRIED OUT ON ALCOHOL EXTRACT



2. Test for alkaloids

- ☐ Concentrate the alcoholic extract on a water bath. Spot the concentrated alcoholic extract on filter paper then Test part of F.P. is dipped in Dragendorff's reagent $KBiI_3$ (Orange spot) against Blank (Don't dip in Dragendorff's reagent (No orange spot).

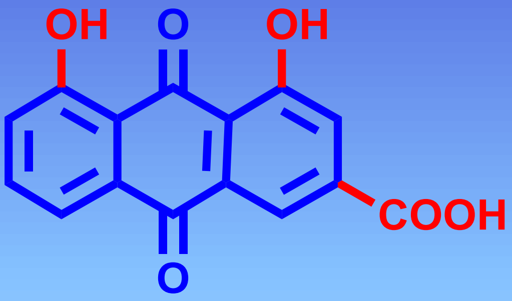




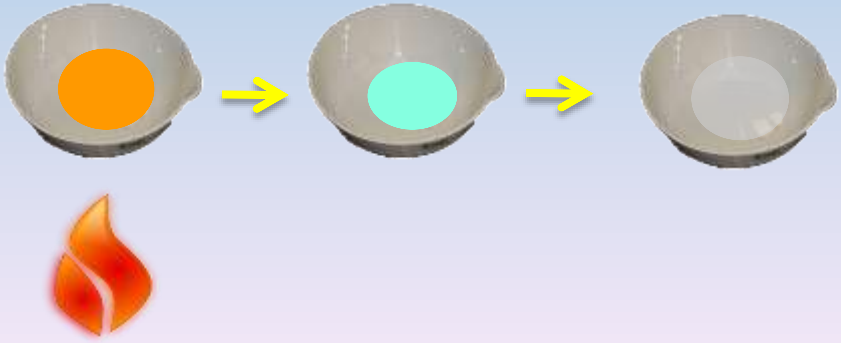
(C) TESTS CARRIED OUT ON ALCOHOL EXTRACT

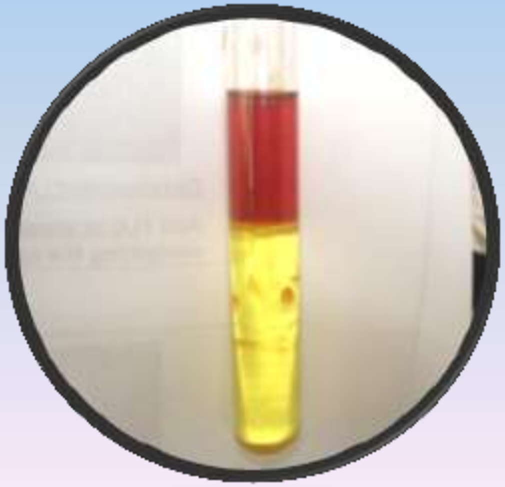
3. Test for anthraquinones

Borntrager's test (Test for free anthraquinones)



- 5ml Alc. Extract evaporated on W.B then cooled.
- Dissolve residue in 5ml $CHCl_3$ then transfer to a test tube + 2ml conc. NH_3 then gentle shaking
- Rose-red colour in aqueous alkaline layer.

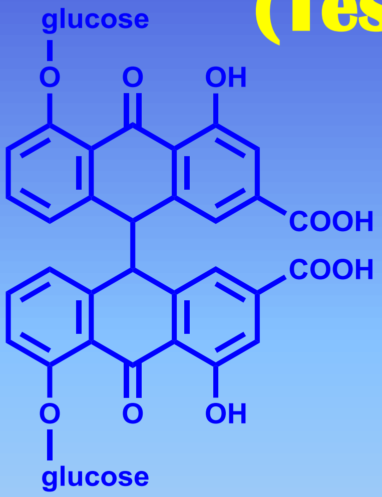


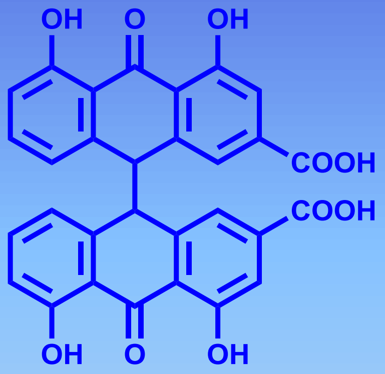


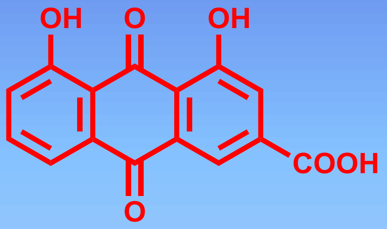
(C) TESTS CARRIED OUT ON ALCOHOL EXTRACT

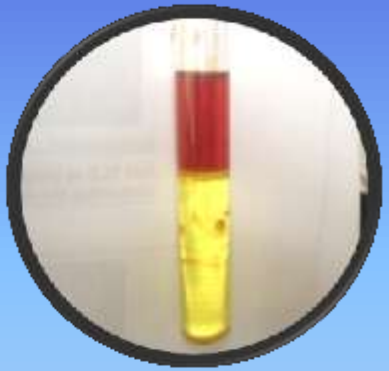
b- Modified Borntrager's test

(Test for combined anthraquinones)







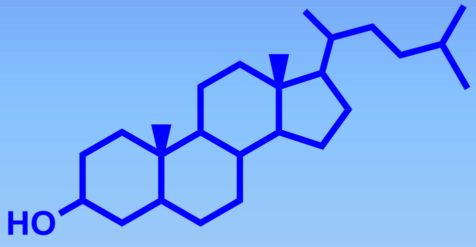


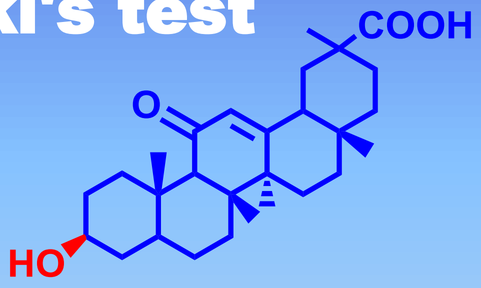
- 5ml Alc. Extract are hydrolyzed by boiling with 1ml conc. HCl to break down the glycoside linkage on water bath.
- Cool then add few drops of H_2O_2 or $Fecl_3$ (oxidizing agent) to break the dimeric linkage and to convert the products to rhein aglycone.
- Extract with 5ml $CHCl_3$
- $CHCl_3$ extract + 2ml conc. NH_3, gentle shaking → rose-red colour in aqueous layer.

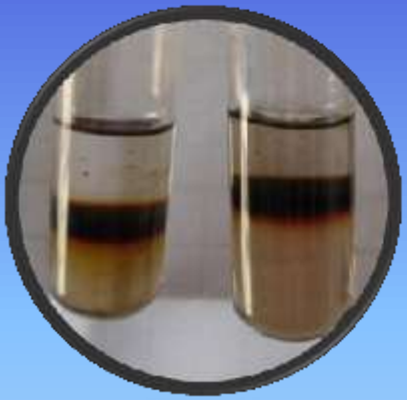
(B) TESTS CARRIED OUT ON ALCOHOL EXTRACT

4.Test for Sterols and /or Triterpenes

Salkowiski's test







- **Evaporate the alcoholic extract in porcelin dish till dryness**
- **Dissolve the residue in 5 ml $CHCl_3$ & transfer into a dry test tube:-**

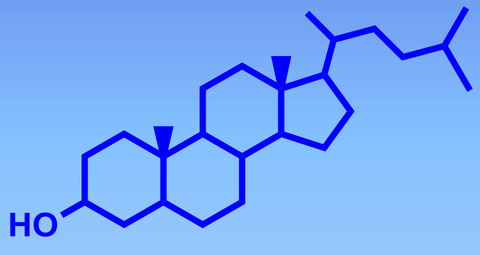
- **2 ml $CHCl_3$ extract + 2ml conc H_2SO_4 → Reddish brown ring.**

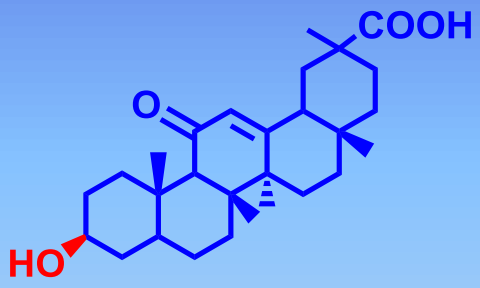
(C) TESTS CARRIED OUT ON ALCOHOL EXTRACT

ALCOHOL EXTRACT FILTERED THROUGH COTTON

Test for Sterols and /or Triterpenes

Liberman's test

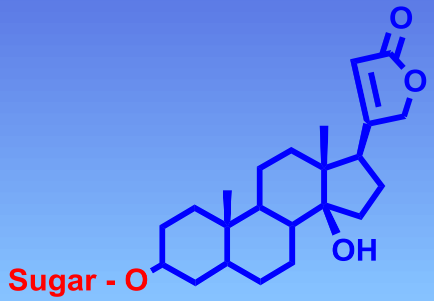




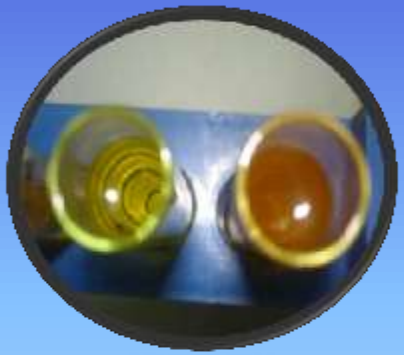
- ☐ Evaporate the alcoholic extract in porcelin dish till dryness
- ☐ Dissolve the residue in $CHCl_3$ & transfer into a dry test tube:-
- ☐ $CHCl_3$ extract + few drops of acetic anhydride + conc H_2SO_4 on the walls of the test tube.
- ☐ Reddish brown ring and green colour in the upper layer (sterols).
- ☐ Violet ring and green colour in the upper layer (triterpenes).

SCREENING OF TABLETS CONTAIN DIGOXIN

Test for cardiac glycosides



Baljet test



- **One tablet grinded and extracted with 5 ml %70 alcohol.**
- **Alc. Extract decanted or filtered through small piece of cotton.**
- **2ml Filtrate + 2ml Baljet reagent (90 ml 1% Picric acid + 10ml 10% NaOH)**
- **Orange – red colour indicate presence of cardiac glycosides.**
- **Compare the colour produced by nesslerization with blank (water + Baljet reagent)**

PHYTOCHEMICAL SCREENING IN QUALITY CONTROL

SCREENING OF RUTA C AND DIOVAN TABLETS

A COMPLAINING CAME TO YOU ABOUT TABLETS CONTAIN RUTIN SAYING: "IT IS NOT EFFECTIVE"

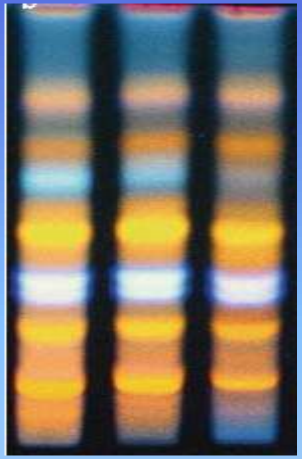
Test for Flavonoids

10% NaOH Test

- ☐ One tablet grinded and extracted with 5 ml 1% HCl
- ☐ 1% HCl Extract decanted or filtered
- ☐ 2 ml Extract + 2ml 10% NaOH → yellow colour (if diosmin or rutin is present)

N.B : Compare with blank (2ml extract + 2ml water)

SCREENING USING TLC



SCREENING USING TLC

TLC: Thin Layer Chromatography

Thin layer of stationary phase (e.g. Silica) spread on aluminium sheet or glass plate.

Advantages:

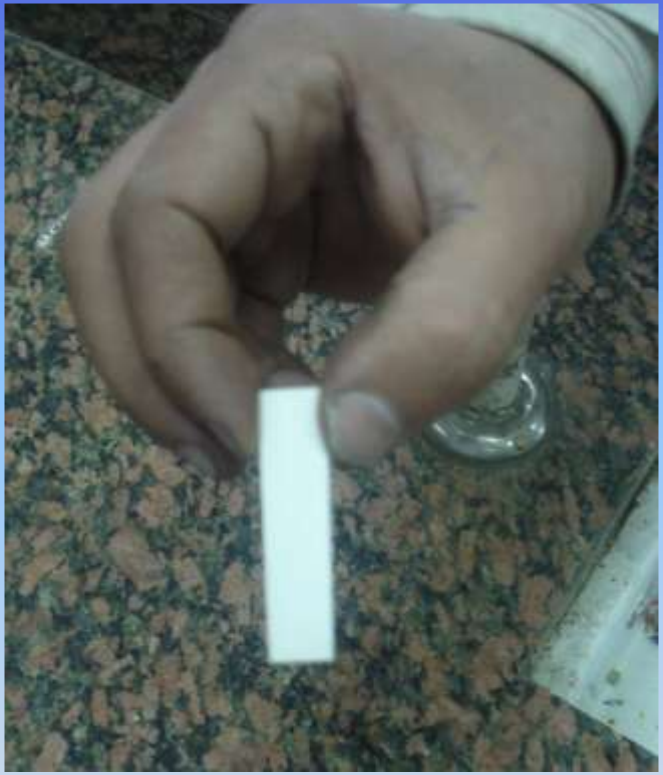
1- Rapid, simple, reliable method.

2- Detection is done using small amounts of sample

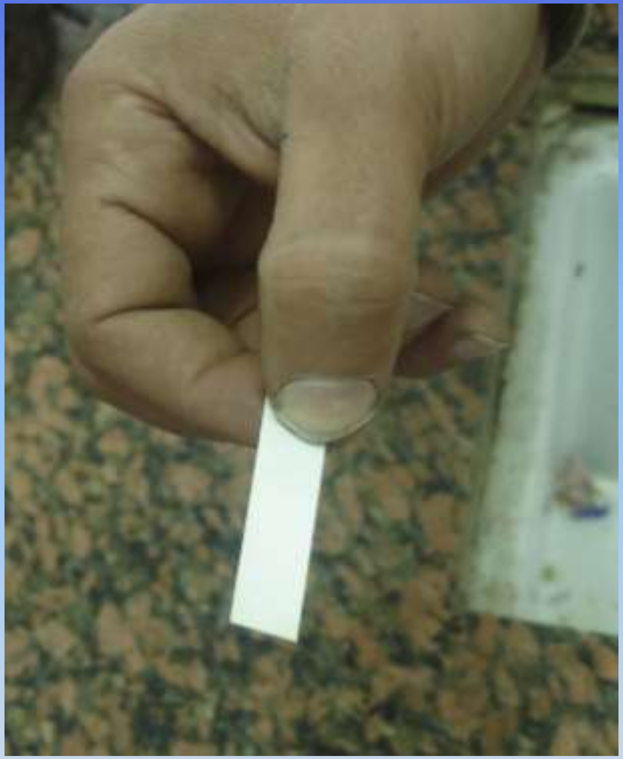
3- The separated spots are more definite than those of paper chromatography.

4- Allow use of drastic spray reagents (e.g. P anisaldehyde/sulphuric acid reagent)

SCREENING USING TLC

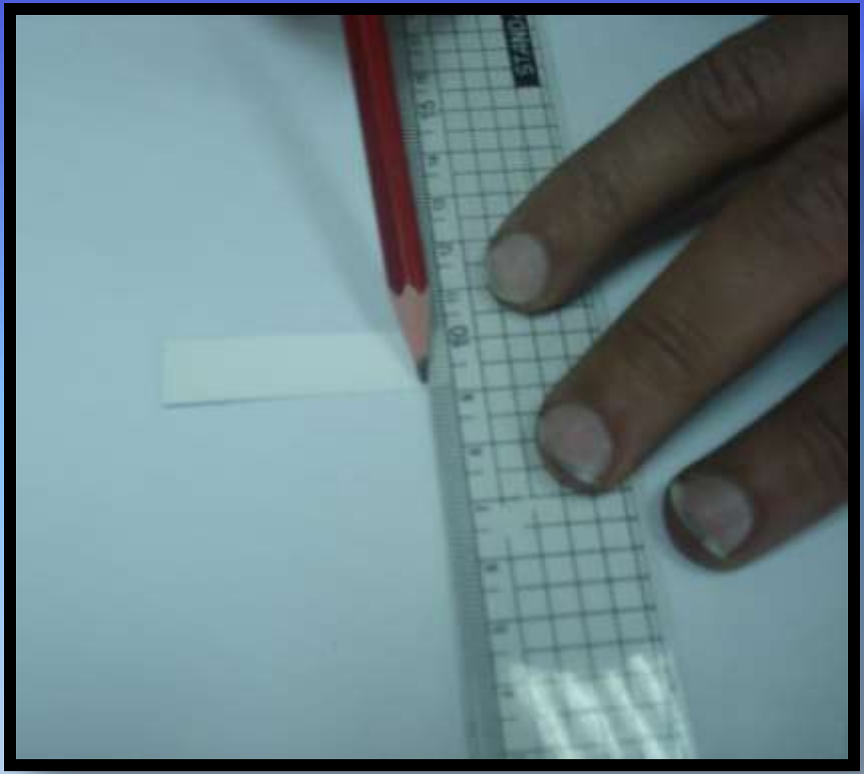


Hold the TLC between your fingertips



Do Not add your finger prints on TLC

SCREENING USING TLC

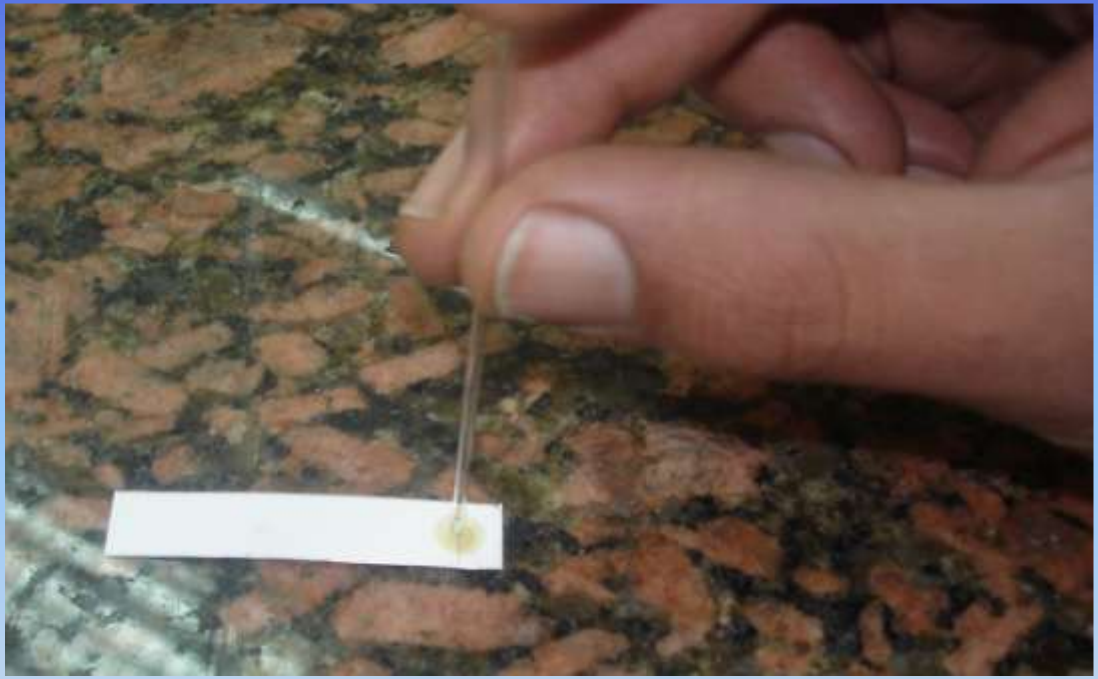


Draw a baseline using fine pencil

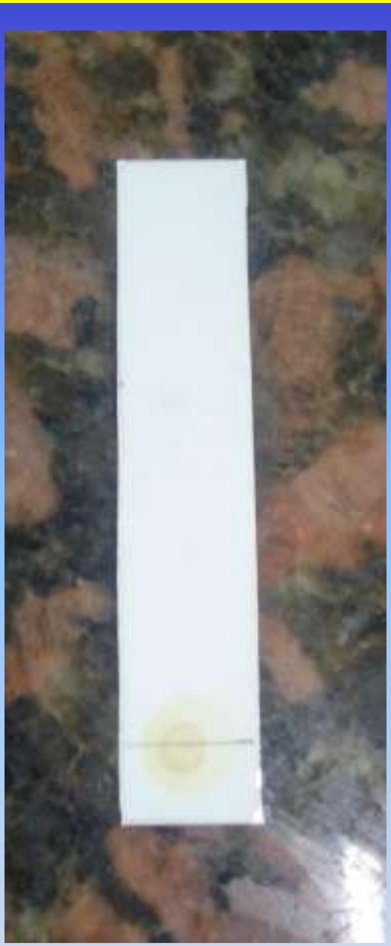
(approximately 1.0 cm from the base)

Do Not scratch TLC

SCREENING USING TLC

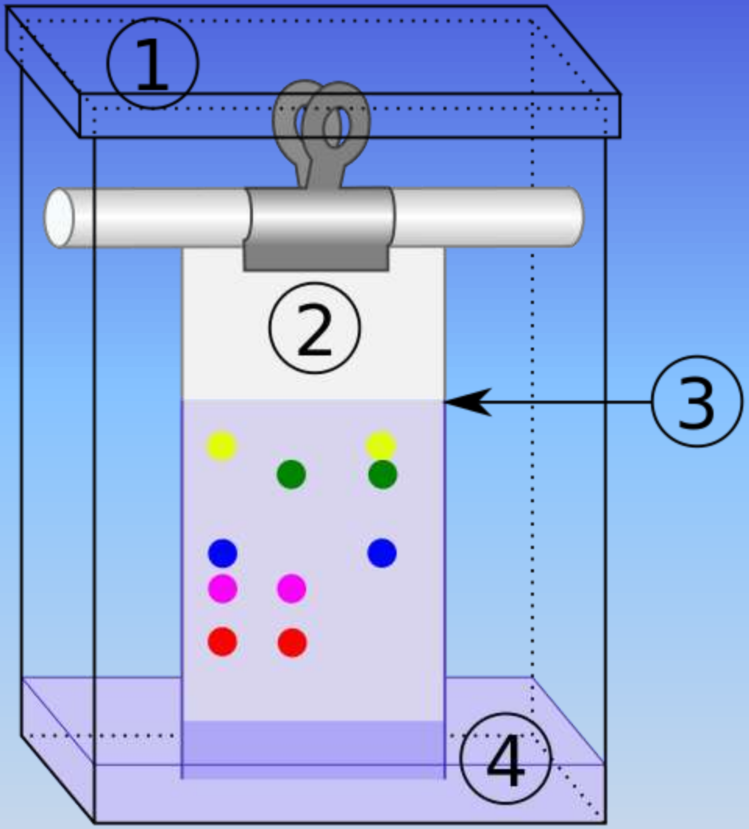


Apply a narrow spot of the alcoholic extract using microdropper



Do Not apply a large amount to prevent expansion of spot

SCREENING USING TLC



Development

After spotting, add TLC in chromatographic jar containing the specific solvent system

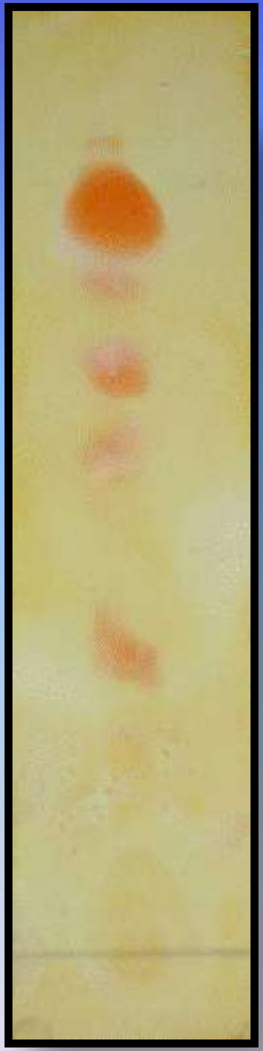
SCREENING USING TLC

After development:

Allow TLC to dry and spray it with the specific spray reagent and examine it as described

SCREENING USING TLC

Sample Screening for Alkaloids



Sample: Extract contains alkaloid

Mobile Phase: $CHCl_3$: MeOH : NH_3

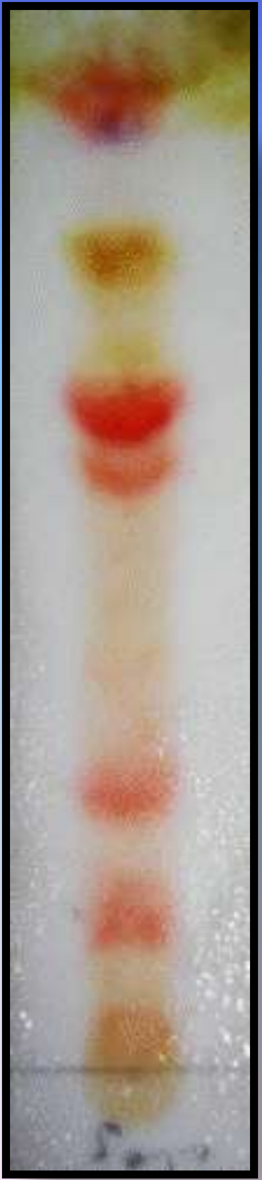
9.5 : 0.5 : 0.1

Detection: Spray with Dragendorff's reagent

(Orange spots)

SCREENING USING TLC

Sample Screening for Anthraquinones



Sample: Extract contains Anthraquinones

Mobile Phase: EtOAc: MeOH: H2O

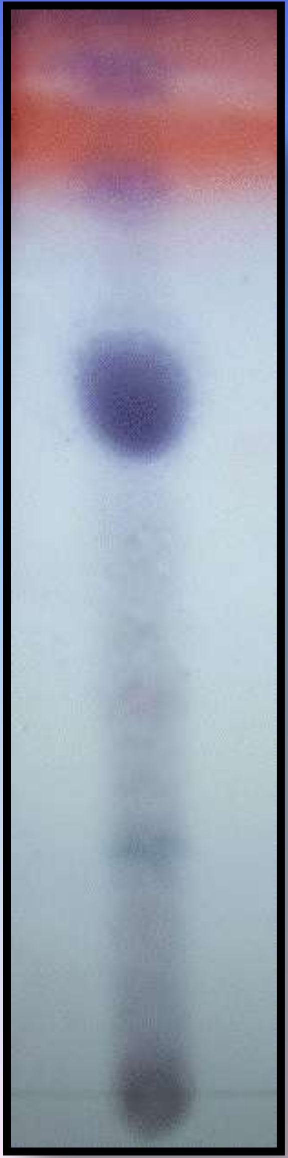
100 : 16 : 13

Detection: Spray with Alcoholic KOH

(Rose red spots)

SCREENING USING TLC

Sample Screening for sterols and triterpenes



Sample : Extract contains Sterols

Mobile Phase: $CHCl_3$:MeOH

9.8 : 0.2

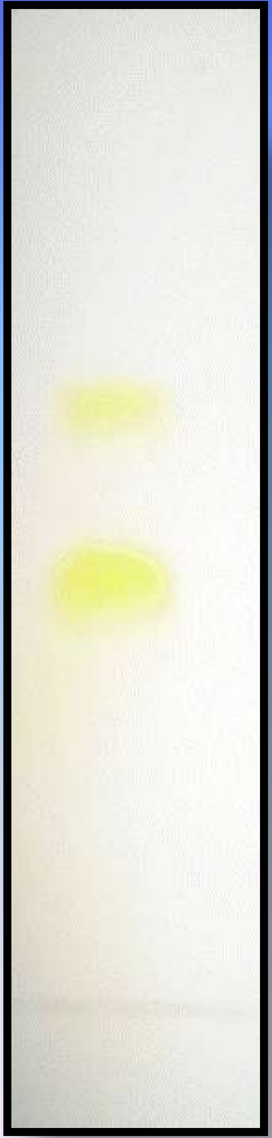
Detection: Spray with P-anisaldehyde/H_2SO_4

Then add in oven for 10 min

(violet or pink spots)

SCREENING USING TLC

Sample Screening for flavonoids



Sample : Extract contains flavonoids

Mobile Phase: EtOAc : MeOH : H_2O

100 : 16 : 13

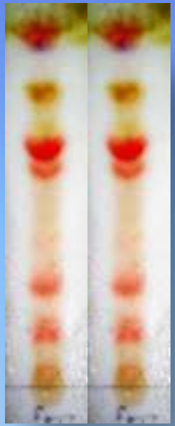
Detection: Spray with $AlCl_3$

(yellow spots in visible light)

SCREENING USING TLC

TLC SCREENING OF TABLETS CONTAIN RHUBARB

Tablets used as laxative were suspected to contain exhausted Rhubarb



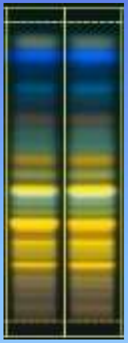
STEPS

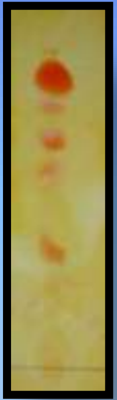
- One tablet grinded and extracted with 5 ml 70% alcohol
- Alcohol extract spotted on TLC plate against Standard Rhubarb extract.
- Develop with Ethyl acetate – Methanol – Water (100:16:13)
- Remove the TLC Plate and carry out air drying
- Dip the TLC Plate in 30% alcoholic KOH Reagent
- Compare the spots numbers and Rfs (TLC Fingerprinting)

SCREENING USING TLC

TLC SCREENING FOR BILACID®TABLETS

Tablets used for renal colic was suspected to contain *Atropa belladonna*



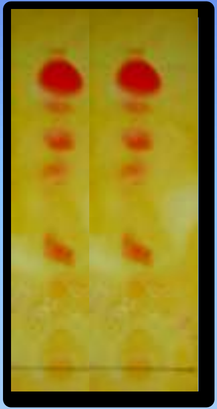


TLC

Screening

TLC

FINGERPRINTING



STEPS

- One tablet grinded and extracted with extracted with 5ml 70% alcohol
- Alcohol extract spotted on TLC plate against Standard *Atropa belladonna* extract.
- Develop with $CHCl_3$ – Methanol – Water (95:5:1)
- Remove the TLC Plate and carry out air drying
- Dip the TLC Plate in Dragendorff's Reagent
- Compare the spots (numbers and Rfs) (TLC Fingerprinting)